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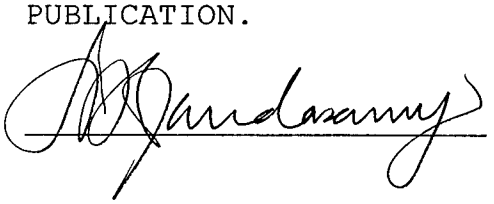

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13. ABSTRACT *(Maximum 200 Words)*

An immunological variant of luteinizing hormone (LH) dependent on two pint mutations in the gene of the LHβ-submit has increased in vitro bioactivity and is detectable in serum with immunofluorometric assays (IFMA). The variant occurs with a frequency of 5--2% in the general population. The altered bioactivity and kinetics of the LH variant are accompanied by changes in LH action and by increased stimulation of the ovarian stromal cells and may be followed by increased production of ovarian testosterone and androstenedione and their estrogen metabolites estradiol and estrone. The study takes advantage of an on-going prospective cohort study of hormones and breast cancer. Serum samples from cohort subjects are being assayed for testosterone, androstenedione, estrone, estrone sulfate, estradiol and SHBG as part of a case-control study nested within the cohort (497 cases and 1,384 controls. In year 2 of the project, we completed laboratory and statistical analyses of the association between breast cancer and the presence of the variant LH. This study was limited to subjects diagnosed at or after age 50. Laboratory analyses of subjects who were younger then age 50 at diagnoses have been completed and statistical analyses are underway.

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Introduction

The production of ovarian steroid hormones in females is dependent on the secretion of pituitary gonadotropins -- follicle stimulating hormone (FSH) and luteinizing hormone (LH). Recently, an immunologically variant form of the LH β-subunit has been identified and described in the laboratory of one of the co-investigators in the present proposal, Dr. I. Huhtaniemi at the University of Turku, Finland. The variant is detectable with a combination of two ultrasensitive immunoassays using monoclonal antibodies (1). Variations in the detectability of LH with the immunoassay may depend on the structural alteration of the epitopes that are recognized by the monoclonal antibodies and indicate that genetic variants of LH exist in some populations. Two point mutations were described in codons 8 and 15 in the LH β-subunit gene (2,3,4) and pedigree analysis confirmed an autosomal recessive mode of inheritance. In codon 8, a TGG to CGG conversion replaces tryptophan (Trp) with arginine (Arg), and within codon 15 a changing ATC to ACC replaces isoleucine (Ile) with threonine (Thr). The latter substitution is of particular interest because it introduces a potential additional glycosylation site in the LH β-subunit, with the potential for increased bioactivity at the LH receptor site (5). In vitro studies have shown that the variant has increased bioactivity in homozygous subjects as compared to those homozygous for normal LH and the in vivo half-life of the variant LH was shorter than for normal LH (6). These data strongly suggest that some individuals carry a more potent form of LH, though with a shorter life span.

The prevalence of the variant LH β-subunit has been estimated among a broad spectrum of world populations. The carrier frequency of the variant LH beta allele varies from a minimum of 71% in US Hispanics to 41.9% in Lapps of northern Finland (7). The variant appears to increase in frequency in populations of Northern Europe, as compared to those of Asia or from tropical climates. In most European populations outside Scandinavia and in Caucasians in the US the variant frequency is around 15% (8).

The finding of a LH polymorphism with potential increased bioactivity suggests that the variant may correlate with changes in gonadal function. To our knowledge, only one study has addressed the relationship of the presence of the variant LH to clinical and hormonal parameters among women with polycystic ovaries as compared to healthy subjects (6). In this study, the variant was appreciably more frequent in obese women with polycystic ovaries than in normal women. Interestingly, among healthy subjects, women with the LH variant had serum estradiol, testosterone and sex-hormone- binding globulin (SHBG) considerably and significantly more elevated than those without the variant. These findings provide preliminary evidence strongly suggestive of a different profile of ovarian sex-hormones among subjects with the LH polymorphism.

Body

In year 1, we conducted two small preliminary studies to clarify specific issues to fine tune study design and management. Later in the year, we completed the first round of laboratory measurements as planned.

First preliminary study. The goal was to determine how well laboratory measurements would predict LH variant status in single samples. The reliability of measurements of LH variants was assessed in the same individual at different sampling times. From the NYWHS database, 20 subjects were identified who had donated samples of blood on two separate occasions. We confirmed that the classification of individuals into categories of LH variants (wild type, heterozygous, homozygous) corresponded exactly in each determination.

Second preliminary study. LH variant measurements were compared in samples that have been stored without ever being thawed and in those that had undergone repeated, complete defrosting. This was useful, as the NYWHS maintains many specimens that are returned from laboratories after the completion of analyses. We compared thawed vs. unthawed samples from 10 subjects and confirmed that the measured values remained unchanged. We concluded that previously thawed samples could be used in place of never thawed ones in subsequent analyses.

First phase of the nested case-control study (Technical Objective 1). The first batch of LH variant analyses relative to study subjects aged 50 or more at the time of diagnosis were completed at the laboratory of Dr. Huhtaniemi in Finland in September 1998. A few additional analyses were repeated during the following weeks to complete missing or incomplete data. The results have been summarized in a manuscript that is ready to be submitted for publication to the journal Cancer Epidemiology Biomarkers and Prevention. Copy of such manuscript is appended (Appendix 1). Overall, these observations do not suggest an association between the presence of LH variant and risk of breast cancer among women who were 50 years or older at the time of diagnosis.

Second phase of the nested case-control study (Technical Objective 1). The second batch of LH variant analyses relative to study subjects aged 49 or less at the time of cancer diagnosis were completed at the laboratory of Dr. Huhtaniemi in Finland in September 1999. A few additional analyses need repeating, owing to technical problems, and are pending. Database preparation and editing is under way. Statistical analyses of the results will be started as soon as the final data are received form the laboratory. Interim analyses suggest a positive, but weak association, between LH variant status and breast cancer diagnosed before age 50. At least one manuscript will be prepared at the completion of statistical analyses, during the winter 1999-2000.

Technical Objective 2 has not been addressed, as yet, owing to a delay in the completion of pertinent biochemical laboratory analyses in the parent study— the NYU Women's Health Study. It is expected that such analyses will be completed by March 2000 and that statistical analyses pertinent to Technical Objective 2 will be conducted shortly thereafter.

Key Research Accomplishments

- Assessment of 11% LHvar allelic frequency in the study population
- Completion of the first case-control study in older subjects (age >49 at diagnosis)
- Evidence of a lack of association between breast cancer and Lhvar genotype in older women

Reportable Outcomes

- Manuscript: Akhmedkhanov A, Toniolo P, Zeleniuch-Jacquotte A, Petterson K, Huhtaniemi I. A genetic variant of luteinizing hormone and risk of breast cancer. Submitted for publication (Appendix 1)

Conclusions

The project has already completed a large portion of its stated objectives. Although observations pertaining to older subjects do not appear to support the basic hypothesis, preliminary results for younger subjects (age less than 50 at cancer diagnosis) are suggestive of a positive

association. If these results are confirmed in final analyses, which are under way, there will be opportunities for further research with potential preventive applications.

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Short Communication

A Genetic Variant of Luteinizing Hormone and Risk of Breast Cancer[1]

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Abstract

A genetic variant of luteinizing hormone (LH), characterized by two point mutations in codons 8 (TGG→CGG) and 15 (ATC→ACC) of the LH β-subunit gene has been recently described. As compared to wild-type LH, this genetic variant appears to have higher *in vitro* bioactivity but shortened circulatory half-life, and it has been reported to affect circulating levels of sex hormones. Our purpose was to determine whether the variant form of LH is associated with altered risk of breast cancer. This hypothesis was addressed in a case-control study nested within a prospective cohort that included 270 cases of breast cancer and twice as many matching control subjects. The study was limited to subjects diagnosed at age 50 or older. Variant LH status in serum was determined by the combination of two immunofluorometric assays using monoclonal antibodies. The frequency of variant LH was similar in breast cancer cases and controls (11.5% versus 10.7%). In conditional regression models, the presence of variant LH was not associated with altered risk of breast cancer (OR = 1.08, 95% CI = 0.66-1.75). Adjustment for potential confounders did not change this estimate. These observations do not appear to support the hypothesis that this particular variant of LH is associated with altered risk of breast cancer diagnosed at age 50 and older.

Introduction

Recently, an immunologically anomalous form of luteinizing hormone has been described in a healthy Finnish woman (1) and, subsequently, in Japan (2). Nucleotide sequencing revealed two missense point mutations in the gene of the LH β-subunit on chromosome 19. One of them (codon 8, TGG→CGG) changes tryptophan to arginine, and the other (codon 15, ATC→ACC) changes isoleucine to threonine (2, 3). Studies of worldwide occurrence of this variant LH revealed a broad variation in frequency, from 55.5% in aboriginal Australians to 0% in Kotas from South India (4, 5). The frequency of variant LH appears to be higher in Scandinavian countries (20-42%), intermediate in Western Europe (15%) and Asia (12-14%), and lowest in Hispanic population in the United States (7%), indicating considerable geographic and racial variation (4).

Analyses of the biological properties of variant LH suggest that the described mutations may alter the physiological function of LH. The mutation at codon 15 is of particular interest because it introduces an additional glycosylation site to the LH β-subunit, with the potential of altered circulatory half-life and bioactivity (6, 7). *In vitro* studies have shown that variant LH has elevated bioactivity in homozygous subjects compared to those homozygous for wild-type LH (8, 9), while *in vivo* half-life of the variant LH in circulation was shorter than for wild-type LH (8). In addition, women heterozygous for the variant LH have somewhat higher serum levels of estradiol, testosterone and sex hormone binding globulin than women without the variant,

indicating alterations of the bioactivity of the variant hormone (10). These findings prompted the suggestion of a more potent form of LH though with a shorter life span.

Several groups reported that variant LH is associated with polycystic ovary syndrome (10-12), characterized by increased levels of circulating LH, increased ovarian androgen production, hyperinsulinemia and multiple cysts in the ovaries, as a result of arrested follicular development. Variant LH may contribute to infertility (2), premature ovarian failure (13, 14) and slow progression of puberty (15). Since LH is an important regulator of steroidogenesis, we hypothesized that the variant form of LH may affect the levels of endogenous sex hormones and subsequent risk of hormone-dependent cancers. A positive association between endogenous estrogens and breast cancer risk in postmenopausal women was observed in our study population (16) as well as in other prospective studies (17).

The aim of this study was to determine if the variant LH genotype is associated with breast cancer in a cohort of mostly Caucasian women from New York City. The present report was concerned exclusively with the risk of cancer occurring at or after menopause. Therefore, subjects diagnosed before age 50 were excluded.

Materials and Methods

Study Population

Between March 1985 and June 1991, the New York University Women's Health Study enrolled a cohort of 14,275 healthy women, aged 34-65 years, at a breast cancer screening center in New York City. Details concerning subject recruitment have been published elsewhere (16, 18). Women who in the preceding 6 months had neither used hormonal

medications nor been pregnant were eligible for enrollment. Blood was drawn prior to breast examination, between 9:00 AM and 3:00 PM, in non-fasting subjects. After centrifugation, serum was divided in 1-ml aliquots and stored at -80°C for subsequent biochemical analyses. Written informed consent was obtained from all cohort members. The study is reviewed and approved annually by the Institutional Board of Research Associates of New York University School of Medicine.

Nested case-control study

Breast cancer cases were identified primarily by active follow-up either at annual mammographic screening (up to 1991) or through questionnaires mailed to each cohort member every 18 months and by computer linkages with tumor registries of the States of New York, New Jersey, Connecticut, and Florida. By January 1995, out of initial cohort of 14,275 women, 113 (0.8%) were lost to follow-up and 180 (1.3%) had withdrawn their collaboration. As of January 1 1995, after 109,111 person-years of follow-up, a total of 417 cases of breast cancer had been identified and confirmed by review of individual clinical and pathological records. Of these, 270 were aged 50 or older at the time of diagnosis and were included in the present nested case-control study. For each case, two controls were selected at random from among cohort members who were alive and free of disease at the time of diagnosis of the case and who matched the case on age at entry (± 6 months), date of enrollment (±3 months), number and dates of subsequent blood donations at the screening clinic.

Laboratory methods

Serum samples from each case and her matched controls were analyzed in the same batch by a laboratory technician who was unaware of their disease status. The LH phenotypes were determined using two different immunofluorometric assays for serum LH determination (Delfia, Wallac Oy, Turku, Finland). The assays used different combinations of monoclonal antibodies (mAb). In the first assay recognizing wild-type LH only, the capture mAb recognizes an epitope in the intact α/β-dimer and the detection mAb recognizes the α-subunit (1). In the second assay recognizing both wild type and variant LH (reference method), two LHβ-specific mAbs were used (19). The ratios of the LH levels measured by these two assays (assay 1/assay 2) fell into 3 separate categories indicating the LH genotype: 1) 1.0-2.1 (normal ratio), the subject has two normal LHβ alleles; 2) 0.5-0.98 (low ratio), the subject is heterozygous for the mutant LHβ gene; and 3) 0-0.03 (zero ratio), the subject is homozygous for the variant LHβ gene (8). The intra- and interassay coefficients of variation of assays 1 and 2 were less than 4% and 5%, respectively, at LH concentrations at and above the lowest standard concentration of 0.6 IU/L of the WHO International Reference Preparation 80/552. Comparison of the LH genotyping by the immunofluorometric assay technique and DNA hybridization assay showed identical results regarding the variant LH and either method can be used as alternatives to determine the LH status (5).

Statistical Methods

The Wilcoxon signed rank test was used to compare continuous variables in cases and controls and the χ^2 test to compare categorical variables. All p values reported are two-sided, and p values less than .05 are considered statistically significant.

Conditional univariate and multivariate logistic regression models were used to assess the association between LH status and breast cancer. Potentially confounding variables were included in multivariate logistic models. They included height, weight, Quetelet index (weight in kilograms divided by height in meters squared), age at menarche (≤ 12, > 12), age at first pregnancy (< 30, ≥ 30, nulliparous), and first-degree family history of breast cancer. All variables (except age at menarche, age at first pregnancy, and family history of breast cancer) were entered as both continuous variables and by quartiles. All analyses were carried out using SAS Version 6.12. Results are expressed as odds ratios (OR) and 95% confidence intervals (CI).

Results

A total of 270 postmenopausal breast cancer cases diagnosed at age 50 or older (229 invasive and 41 non-invasive) and 540 matching control subjects were included in the analysis. Some characteristics of the study group are given in Table 1. The majority of study subjects (72%) were Caucasian, 8% were African-American, and 3% were Hispanic. This ethnic composition reflects the characteristics of the patient population at the screening clinic at the time of recruitment. The median age at diagnosis of breast cancer was 61 years and the median period between initial blood donation and diagnosis was 2.3 years. Compared to controls, case subjects were more likely to report a prior

benign breast condition (62.4% versus 51.2%, $p < 0.005$), had a higher weight (mean, 69 versus 67 kg, $p < 0.006$) and Quetelet index (mean, 25.9 versus 25.5, $p < 0.05$). Breast cancer cases also had earlier age of menarche (mean, 12 versus 13 years, $p = 0.06$) and later age at first pregnancy (mean, 26 versus 25 years, $p = 0.07$).

Table 2 shows the distribution of variant LH in breast cancer cases and controls. Out of 810 subjects included in the analysis, 89 had low assay 1/assay 2 LH ratio (83 heterozygous and 6 homozygous subjects) corresponding to a variant LH prevalence rate of 11.0%. There was no significant difference in the frequency of LH variant between cases and controls (11.5% versus 10.7%, respectively, $p = 0.75$). Among cases, the median age of breast cancer diagnosis was similar in women with wild-type LH (61.4 years) and variant LH (60.2 years).

In logistic regression analyses, we computed odds ratios for breast cancer associated with LH status. The presence of variant LH status (heterozygotes plus homozygotes) was not associated with an apparent increase in breast cancer risk (OR = 1.08, 95% CI = 0.66 - 1.75). Adjustment for height, weight, Quetelet's index, age at menarche, age at first pregnancy, history of a prior benign breast condition and first-degree family history of breast cancer did not change this estimate.

Discussion

This study was undertaken to determine whether the recently discovered genetic variant of LH, characterized by higher *in vitro* bioactivity in the stimulation of steroidogenesis (8, 9) and higher circulating levels of estradiol and testosterone (10), but shorter circulatory half-life (8), is associated with breast cancer risk. Previously it has been

shown that variant LH may be associated with clinical conditions, including polycystic ovary syndrome (10, 11), menstrual disorders (13, 14), and delayed puberty (15), but not, to our knowledge, with breast cancer. In a cohort of mostly Caucasian women, we found no evidence that variant LH genotype is associated with risk of breast cancer diagnosed at age 50 and older.

The major limitation of the study was its relatively small sample size, especially considering the low prevalence of variant LH (heterozygous and homozygous) in our cohort (11%), as compared to previous observations in Scandinavia and Western Europe (4).

Only 6 out of 810 subjects (2 cases, 4 controls) were homozygous for the variant LH. Even though these numbers are small, an identical prevalence of homozygosity in cases and controls does not give substance to the argument that the effect of variant LH on breast cancer is more pronounced in homozygous than in heterozygous subjects.

In conclusion, the results of the present study do not appear to support the hypothesis that the variant form of LH is associated with an altered risk of breast cancer diagnosed at age 50 and older. It is conceivable that high bioactivity coupled with short half-life could compensate for each other with no apparent effect on breast cancer risk.

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Footnotes

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[3] The abbreviations used are: LH, luteinizing hormone; UK, United Kingdom; mAb, monoclonal antibodies; IU/L, international units per liter; OR, odds ratio; CI, confidence interval.

Table 1 Selected characteristics of breast cancer cases and controls, New York University Women's Health Study, 1985-1994[a]

Characteristic	Breast Cancer Cases (n = 270)	Controls (n = 540)	P^b
Age at blood donation	58 (44-68)	58 (43-68)	0.87
Age at menarche	12 (9-17)	13 (8-17)	0.06
Ever pregnant (%)	79.8	83.1	0.28
Age at first full-term pregnancy	26 (16-41)	25 (16-43)	0.07
Breast cancer in first degree relative (%)	21.9	22.0	0.95
Prior benign breast condition (%)	62.4	51.2	<0.005
Height (cm)	162.4 (150-183)	161.6 (145-183)	0.19
Weight (kg)	69 (47-123)	67 (45-141)	<0.006
Quetelet index (kg/m²)	25.9 (17.0-43.5)	25.5 (17.0-54.8)	<0.05

[a] Means (range), unless otherwise specified.
[b] χ^2 test for proportions and Wilcoxon two-sample test for means.

Table 2 Luteinizing hormone status of breast cancer cases and controls, New York University Women's Health Study, 1985-1994

LH Status	Breast Cancer Cases (n = 270)	Controls (n = 540)	OR (95% CI)
Normal LH (wild-type)	239 (88.5%)	482 (89.3%)	-
Variant LH (heterozygotes)	29 (10.7%)	54 (10.0%)	1.08 (0.65 – 1.79)
Variant LH (homozygotes)	2 (0.7%)	4 (0.7%)	1.01 (0.13 – 6.42)
Variant LH (heterozygotes + homozygotes)	31 (11.5%)	58 (10.7%)	1.08 (0.66 – 1.75)

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
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ATTENTION OF:

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2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

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PHYLIS M. RINEHART
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